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Bioenergy

06 MAR 2012

Dr. Patrick O. Bradshaw
Program Manager
AFOSR/RSL
Air Force Research Laboratory

Report Documentation Page

Form Approved
OMB No. 0704-0188

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1. REPORT DATE **06 MAR 2012**	2. REPORT TYPE	3. DATES COVERED
4. TITLE AND SUBTITLE **Bioenergy**		5a. CONTRACT NUMBER
		5b. GRANT NUMBER
		5c. PROGRAM ELEMENT NUMBER
6. AUTHOR(S)		5d. PROJECT NUMBER
		5e. TASK NUMBER
		5f. WORK UNIT NUMBER
7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES) **Air Force Research Laboratory,Wright Patterson AFB,OH,45433**		8. PERFORMING ORGANIZATION REPORT NUMBER
9. SPONSORING/MONITORING AGENCY NAME(S) AND ADDRESS(ES)		10. SPONSOR/MONITOR’S ACRONYM(S)
		11. SPONSOR/MONITOR’S REPORT NUMBER(S)

12. DISTRIBUTION/AVAILABILITY STATEMENT
Approved for public release; distribution unlimited.

13. SUPPLEMENTARY NOTES
The original document contains color images.

14. ABSTRACT

15. SUBJECT TERMS

16. SECURITY CLASSIFICATION OF:			17. LIMITATION OF ABSTRACT	18. NUMBER OF PAGES	19a. NAME OF RESPONSIBLE PERSON
a. REPORT **unclassified**	b. ABSTRACT **unclassified**	c. THIS PAGE **unclassified**		**30**	

2012 AFOSR Spring Review
Portfolio Overview

NAME: Patrick O. Bradshaw, Ph.D.

BRIEF DESCRIPTION OF PORTFOLIO:

• **Bioenergy** is a program that characterizes, models and explains the structural features, metabolic functions and gene regulatory mechanisms utilized by various biological systems to capture, transfer, convert, or store energy for the purpose of producing renewable biofuels and improving the power output of biofuel cells. (~80% of portfolio)

Sub-Areas: (1) BioSolar Hydrogen, (2) Algal Oil (3) Artificial Photosynthesis, and (4) Biofuel Cells (Microbial and Enzymatic)

• **Photo-Electro-Magnetic Stimulation of Biological Responses** is a beginning program that characterizes, models and explains the stimulatory and inhibitory responses of biological systems to low-level exposures of photo-electro-magnetic stimuli. Potential long-term benefits may include accelerated recovery from mental fatigue and drowsiness, enhanced learning and training, and noninvasive treatment of traumatic brain injuries. (~20% of portfolio)

Visionary Transformational AF Capabilities

Bioenergy:

- **Biofuel Produced from CO_2, H_2O and Sunlight:**
  - Algal systems biology data used to bioengineer lipid biosynthetic pathways in microbes or to create novel synthetic pathways in artificial solar fuel systems

- **Portable H_2 Fuel Generated from H_2O or Cellulose:**
  - Cheap, self-healing inorganic catalysts split water into H_2 and O_2
  - Engineered photosynthetic microbes produce H_2 fuel

- **Compact Power from Ambient Biomass:**
  - Efficient electron transport coupled with unique electrode architectures enhance power and energy densities of biofuel cells

Photo-electro-magnetic Stimulation of Bio-Responses:

- **Electromagnetically Enhanced Cognition, Protection and Healing:**
  - low-level exposure with photo-electro-magnetic stimuli enhance cognitive functions, bio-molecular repair and bio-resiliency

Overview of Topic Areas 3003P

Bioenergy: Alternative Energy

- **Biofuels—Macro-scale Energy**
  - ↘ **Biosolar Hydrogen**
  - ↘ **Algal Oil for Jet Fuel**
  - ↗ **Artificial Biology**

- **Biofuel Cells—Micro-scale Energy**
  - → **Enzymatic Fuel Cells**
  - → **Microbial Fuel Cells**
  - → **Artificial Photosynthesis**

Sun → **Photosynthesis** → **Fuel** $\xrightarrow{H2}$ Fuel Cells → Small Vehicles, portable power

Natural to Artificial

Fuel $\xrightarrow{Cellulose}$ **Biofuel Cells** → MAV

Future Direction

- ↗ **Photo-Electro-Magnetic Stimulation of Biosystems**
- ↗ **Biomarkers, Physiological responses and toxicology**
- ↗ **Artificial Biology – explore non coding genetic information**

Bioenergy:
A Progressive Research Strategy

Natural to Artificial

Sun → Photosynthesis → Fuel → Biofuel Cells → POWER

Generation	1st	2nd	3rd	4th
System Type	Natural Biosystems ↔	Optimized Natural Biosystems ↔	Hybrid Systems ↔	Artificial Systems
	↕	↕	↕	↕
Basic Research Type	Characterization Mechanisms Models ↔	Metabolic/ Protein Engineering ↔	Synthetic Biology ↔	Chemistry & Materials Science
Disciplinary Inputs	Biology		Chemistry	
	← Math ↔	Physics ↔	Engineering →	

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Challenges, Opportunities and Breakthrough Examples

Natural Systems Research:

Challenge: Explain gene regulatory mechanisms of metabolic pathways and networks

Payoffs: - potentially economical viable biofuels
- enhanced energy density of microbial fuel cells (MFC)

Challenge: Understand mechanisms and kinetics of enzyme-catalyzed reactions

Payoffs: - enhanced energy density of enzymatic fuel cells (EFC)
- sustained oxygen-tolerant hydrogen production by photosynthetic microbes

Artificial Systems Research:

Challenge: Discover/fabricate cheap, durable synthetic materials that mimic the enzymatic or structural functions in natural energy systems

Payoffs: - cheap water-splitting catalysts as platinum replacements in H2-generating devices
- enhanced power and energy densities for EFC

Challenge: Integrate and assemble nano-scale inorganic/organic/bio-materials

Payoffs: - ordered enzyme alignments for enhanced power densities in EFC
- enhanced electron transport and power density in biofuel cells
- light is harvested and split in artificial photosynthetic solar fuel generator

Photosynthesis, Systems Biology and Metabolic Engineering for the Production of Biofuels

Microalgae & Cyanobacteria Make Hydrogen, Lipids & Sugars

Light Reactions PSI and PSII

light → chlorophyll

$2\ H_2O$ → water-splitting enzyme

$4\ e^-$

$4\ H^+$

Dark Reactions

CO_2

carbon-fixing enzyme

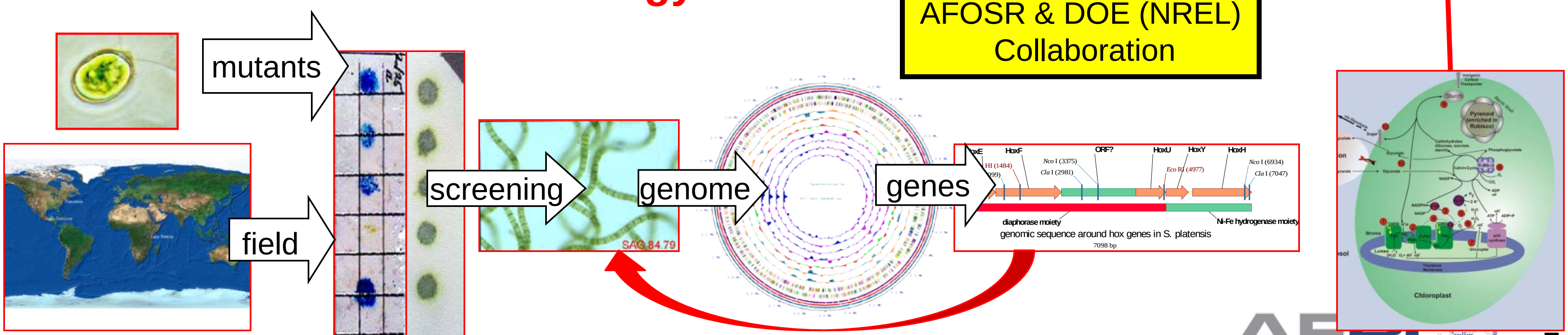
Triglyceride (Oil) Lipid Synthesis → Jet Fuel

Sugar/Cellulose Synthesis → Ethanol

H_2-generating hydrogenase enzyme → H_2

Three Key Biocatalysts

Overview of Research Strategy



Biosolar Hydrogen

(MURI and Core Funding)

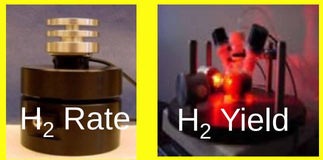
Bio-Solar Hydrogen Production
Eight Labs Including AFRL & DOE

Objective:

Light + 2 $H_2O \rightarrow O_2$ + 2 H_2 (H^+/e^-)

- Obtain knowledge of the basic scientific principles governing H_2 production in microalgae and cyanobacteria
- Genetically engineer pathways to improve the H_2 producing capacity of these phototrophs

H_2 Detectors



- Electrode consumes H_2
- Extended spectral range
- Increased light source intensity 500X with LED

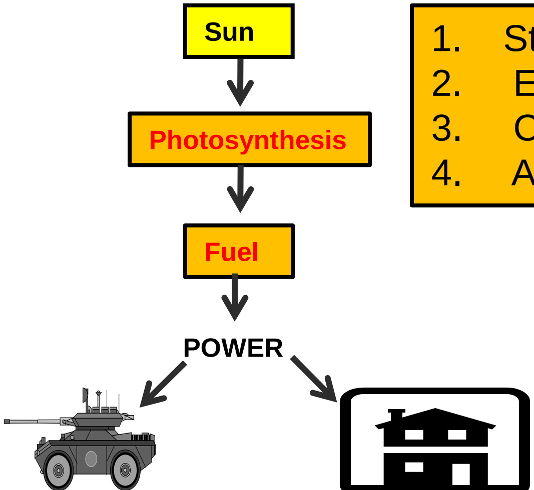
Technical Approaches:

- Bio-prospecting new strains & species
- New H_2 detection & analytical methods
- Stress responses and H_2 production
- Systems biology and pathway analyses
- Genetic engineering of pathways

Accomplishments:

- Developed techniques for high throughput screening of H_2-producing phototrophs
- Identified physiological factors for increasing rates & yields of cellular H_2 production
- Engineered metabolic pathways with increased production of H_2

DoD Benefit:

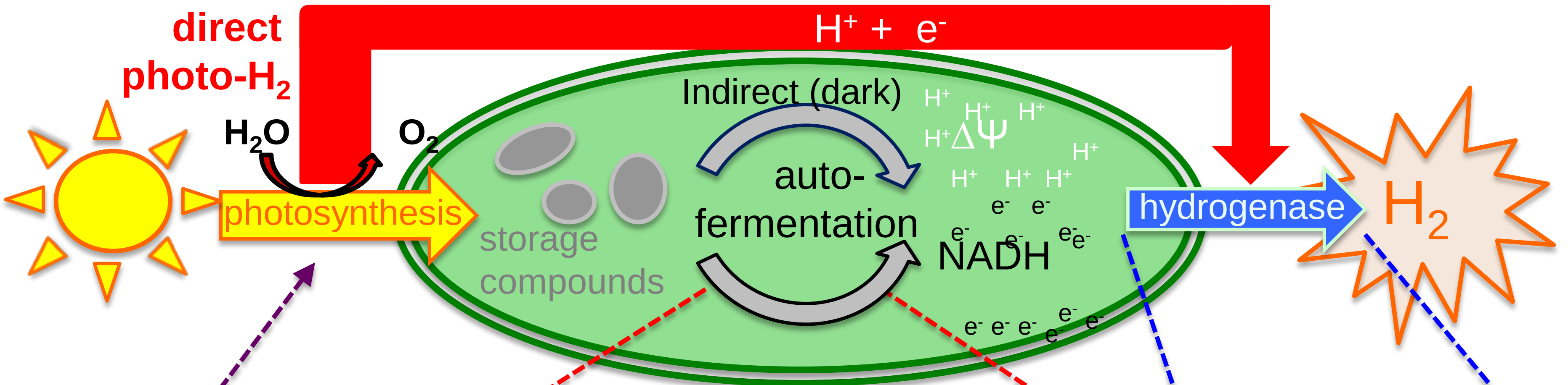


1. Stable fuel supply & price
2. Energy independence
3. Carbon neutral
4. Anti-climate change

BioSolar H_2 Cyanobacterial Metabolism

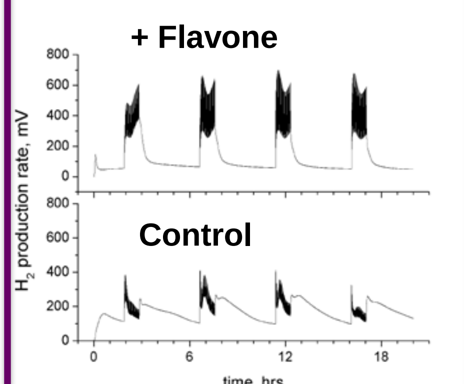
Improving Cellular Fuel Production Efficiency

Dismukes (Rutgers)



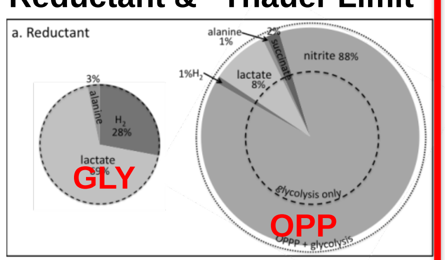
Targets for Protein Engineering

Channeling reductant flux through one of two NADH enzymes increases photo-H_2

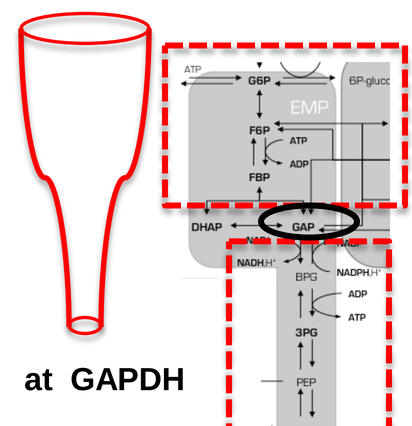


Revealed NO_3^- master switch between glycolysis (GLY) & oxidative pentose phosphate (OPP)

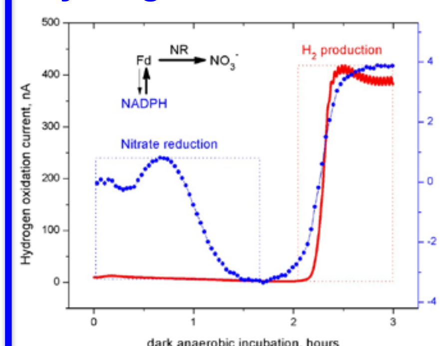
Reductant & "Thauer Limit"



Identified the metabolic bottleneck in glycogen fermentation

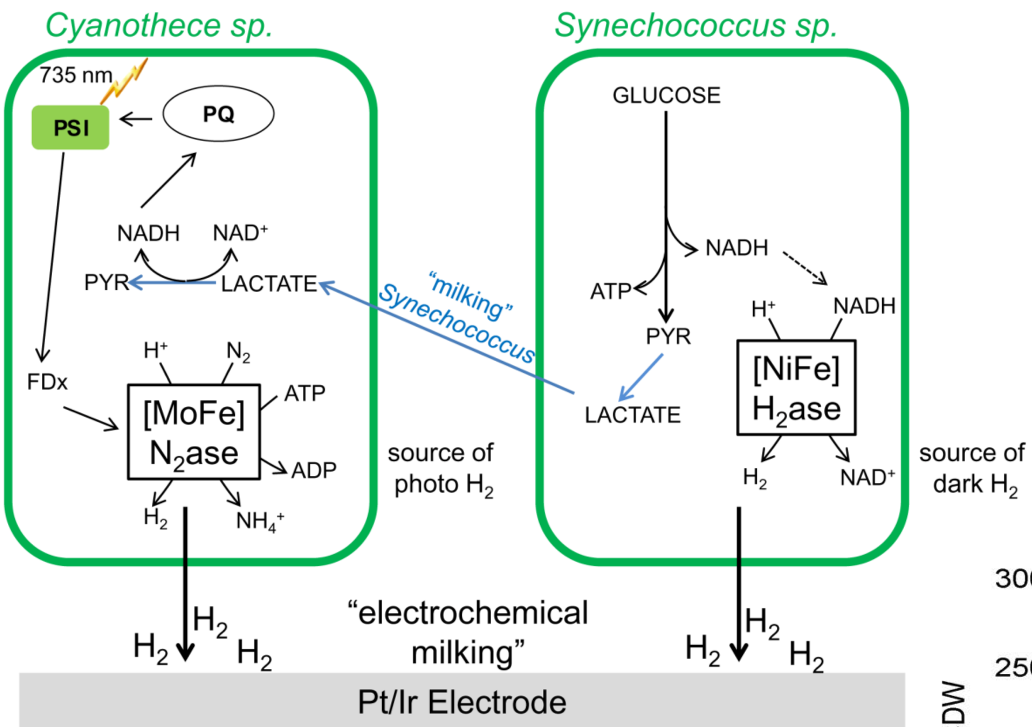


NADH is reductant for phase II H_2 and NAD+ is feedback inhibitor of hydrogenase



“Milking” More H_2 by Co-Fermentation

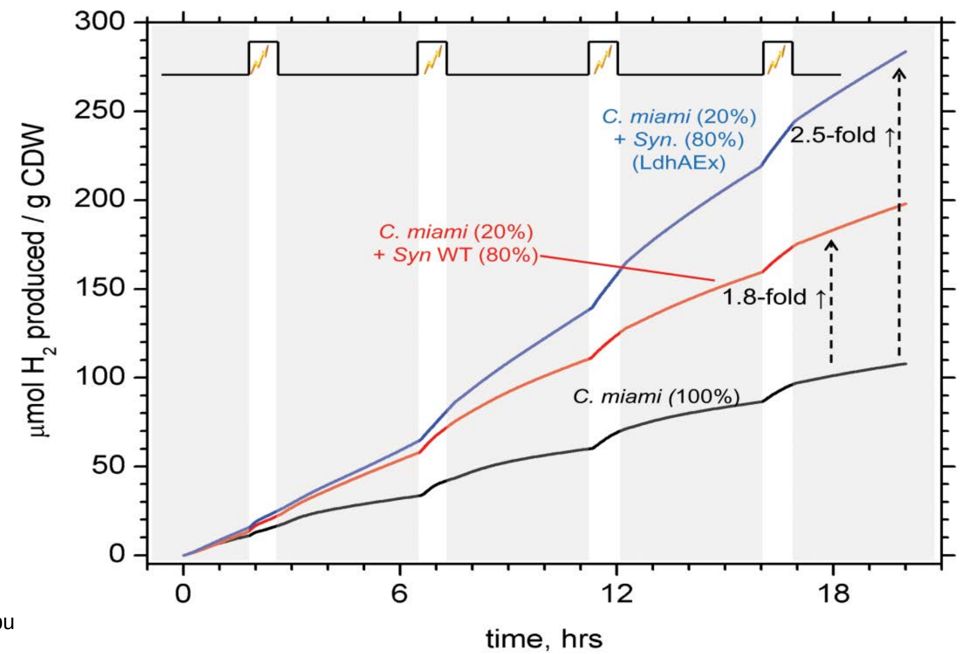
PI: G. C. Dismukes ***Spring Review FY12***



Separate Growth

Cyanothece sp. “photo” fermenter + *Synechococcus* sp. “dark” fermenter

Co-Fermentation



*Rate of Dark+Photo H_2 ↑ from *Cyanothece* is limited by intracellular reductant glycogen

**Syn. WT* excretes reductant as lactate which stimulates 2x H_2 from mixed cultures with *Cyanothece*

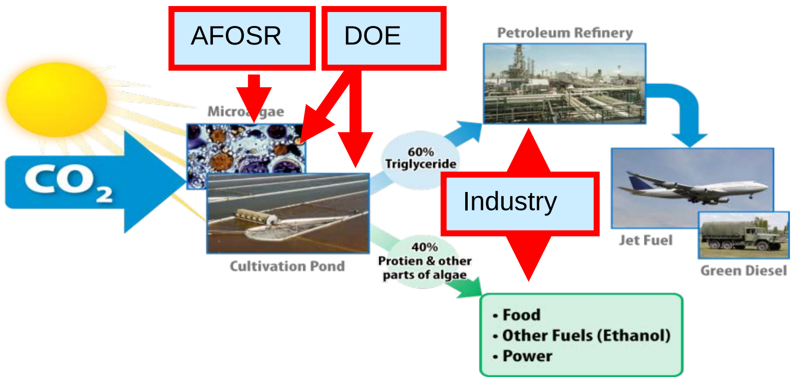
**SynLdhAEx* Over-expression strain excretes more lactate than *Syn WT* and stimulates H_2 even more by 2.5x

Algal Oil

Algal Oil

Ten Labs Including DOE and USAFA

Objective: Gain knowledge of basic algal biology needed to engineer and enhance photosynthetic and lipid biosynthetic pathways



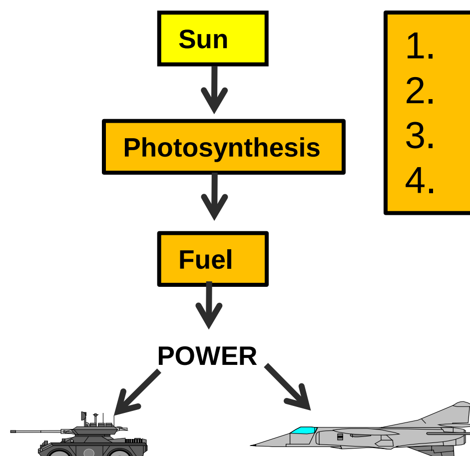
Technical Approach:

- Partner with DOE's National Renewable Energy Lab
- Bioprospect for new lipid-producing algal strains
- Optimize light capture and photosynthetic efficiency
- Optimize environmental factors for lipid biosynthesis
- Use systems biology ("omics") to map lipid pathways
- Identify genetic targets and model metabolism
- Build genetic tools for enabling algal bioengineering

Accomplishments:

- Screened1200 algal strains for oil yield and identified 50 candidate strains for future studies
- High pH raises oil yields further in NO_3-stressed cells
- Transformed carbonic anhydrase into algal genome, resulting in CO_2 availability and enhanced growth rate
- Cell cycle arrest or silica starvation elevates lipid production in brown algae (diatoms)
- Identified proteins involved in forming intracellular lipid droplets and in controlling their storage capacity

AF Benefit:

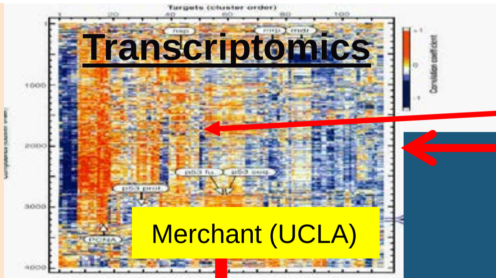


1. Stable fuel supply & price
2. Oil independence
3. Carbon-neutral
4. Anti-climate change

Systems Biology for Algal Lipid Pathway Analyses: A 7 Lab Collaboration

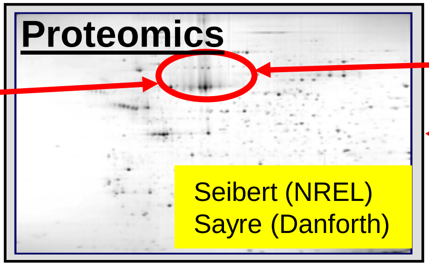
Objectives: Next generation RNA Sequencing technologies are used to compare gene expression profiles in lipid- and non-lipid-producing algae

APPROACH



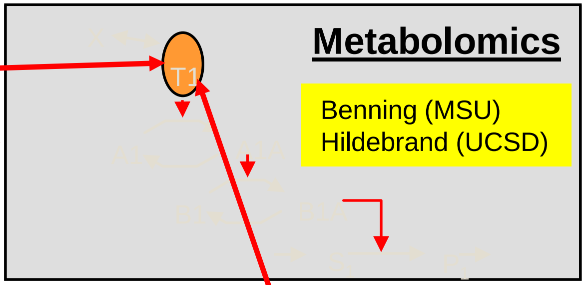
Transcriptomics

Merchant (UCLA)



Proteomics

Seibert (NREL)
Sayre (Danforth)



Metabolomics

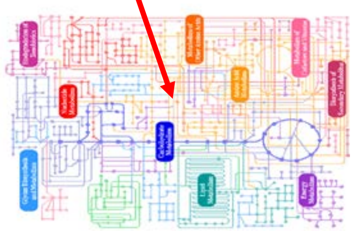
Benning (MSU)
Hildebrand (UCSD)

Bioinformatics:
Data collection & processing

Pellegrini (UCLA)

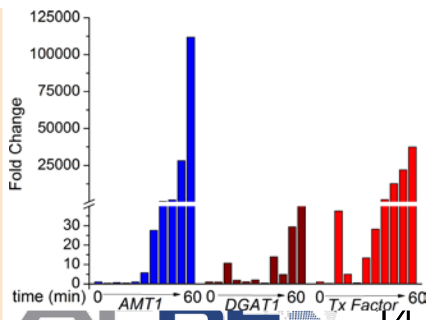
Computational Biology:
Mathematical modeling & pathway mapping

Rabinowitz (Princeton)



Recent Findings:

- 3 time-course experiments analyzed by RNA-Sequencing: from 0 to 48 h
- *DGAT1, triglyceride synthesis enzyme,* is induced early in the time course
- A transcription factor, *NRTF1*, is co-expressed with *DGAT1*
- Developed a web-based protein function annotation tool for algal genomes (http://pathways.mcdb.ucla.edu/chlamy/)

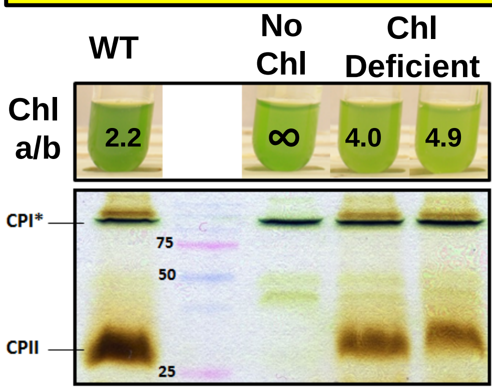


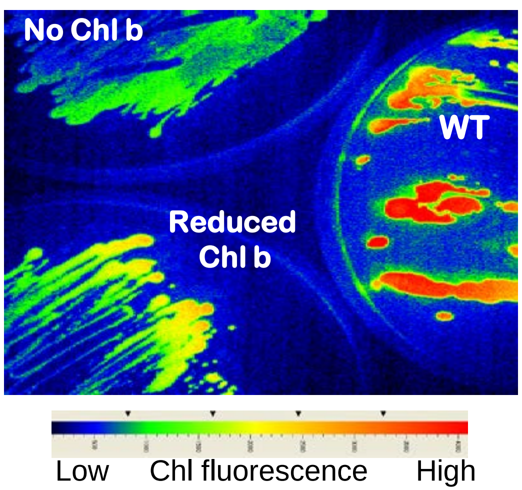
Enhanced Photosynthetic Efficiency & Algal Growth by Optimizing Light Harvesting Antennae Size

Richard Sayre (Danforth Plant Science Center)

Transgenic algae with reduced Chl b have:

1) Reduced antennae size
2) Reduced steady state fluorescence

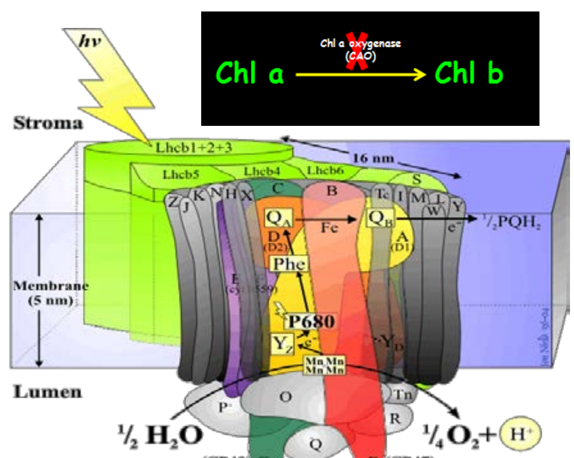


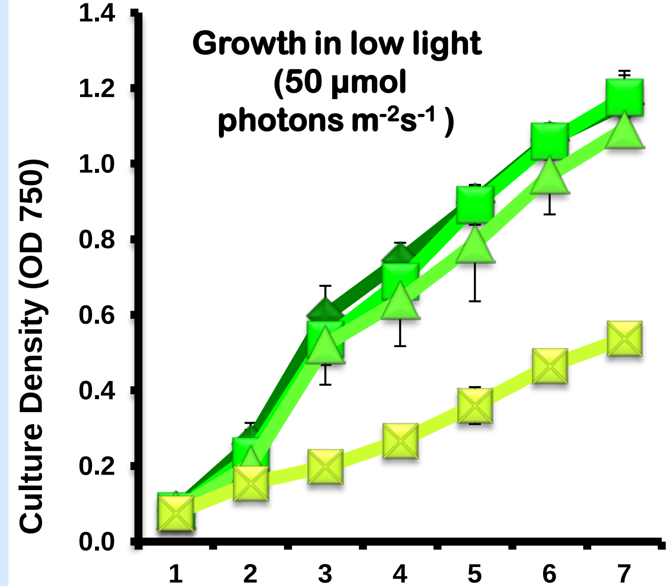


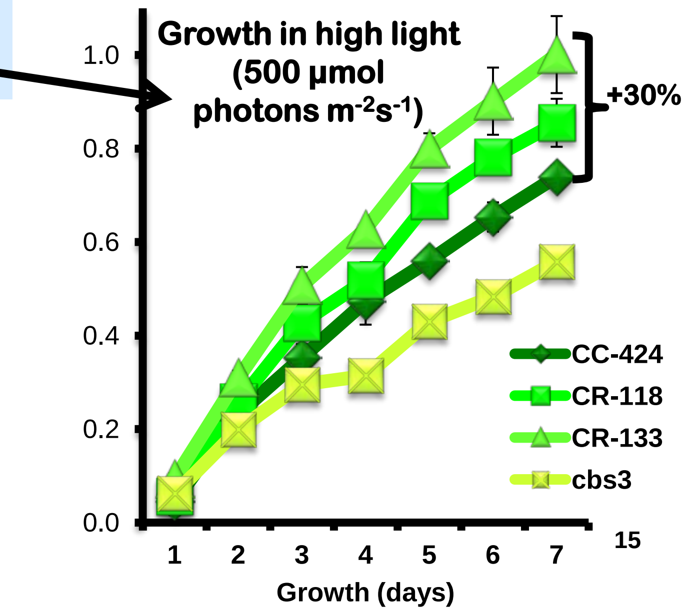
FACT:
At full sunlight 75% of the captured energy is given off as fluorescence or heat.

HYPOTHESIS:
Reducing the antennae size optimizes energy transfer between the antennae and reactions centers

RESULT:
Reductions in Chl b levels reduced the antennae size resulting in a 30% increase in biomass yield at high light intensities relative to wild type







Enzymatic Fuel Cell

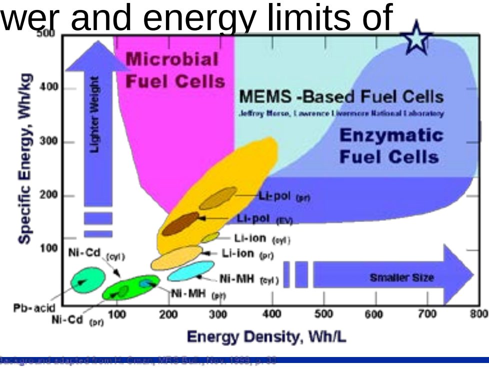
Fundamentals and Bioengineering of Enzymatic Fuel Cells: Seven Labs Including AFRL

Objectives:

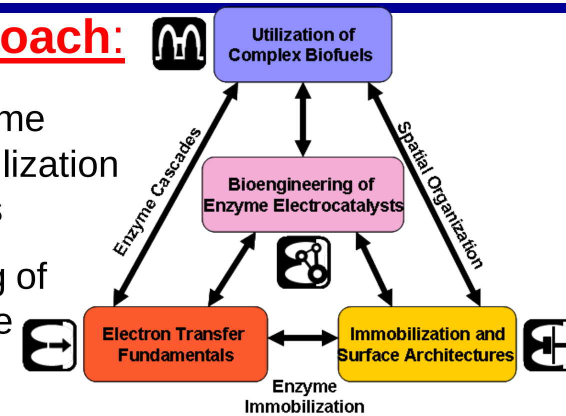
(1) Exploit biochemical reactions for converting chemical to electrical energy and for generating power from fuels readily available in the environment.

(2) Estimate the specific power and energy limits of enzyme fuel cells to define potential powering uses

(3) Transition technology towards sub-miniature sustainable mobile power sources



Technical Approach:



- Provide multi-enzyme cascades for full utilization of complex biofuels
- Protein engineering of enzymes to improve bioelectrocatalysts
- Establish mechanisms of electron transfer
- Design and fabricate novel electrode architectures for enhanced performance

Accomplishments:

- Developed multi-enzyme cascades for complete oxidation of biofuels, enhancing energy density
- Modeling identified major obstacles in multi-step enzyme catalysis—electrode surface area and co-factor (NAD) instability
- Engineered enzymes to self-assemble into conducting hydro-gels and broadened their specificity to accept both NAD & NADP
- Determined O_2 binding site in multi-copper oxidases

DoD Benefit:

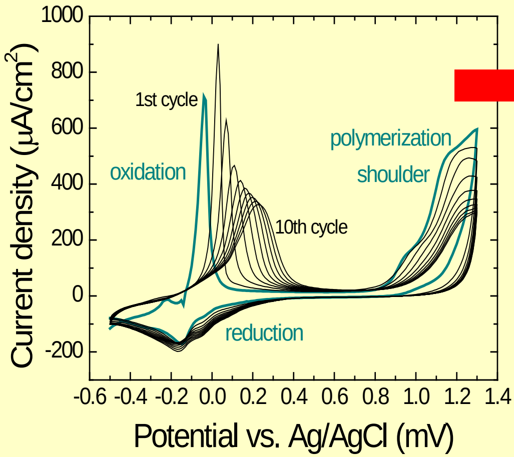
Energy technology platform for scalable power generation. Particularly useful at miniature and micro-levels. Enabling technology for sensors and MEMS devices

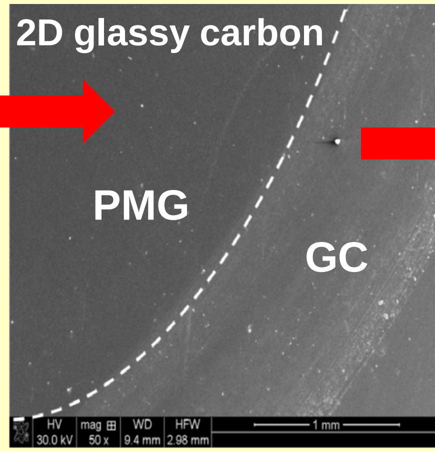
Integrated Enzymatic Biofuel Cell

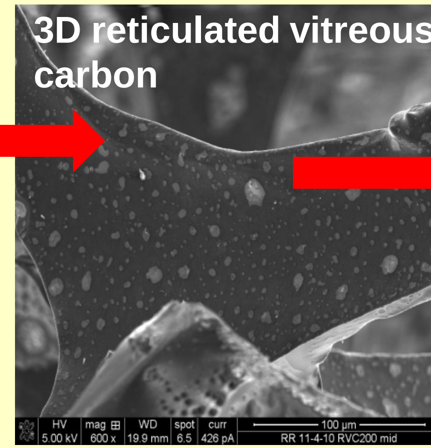
Atanassov (UNM)

Deposition and characterization of poly-(methylene green) catalysts for NADH oxidation

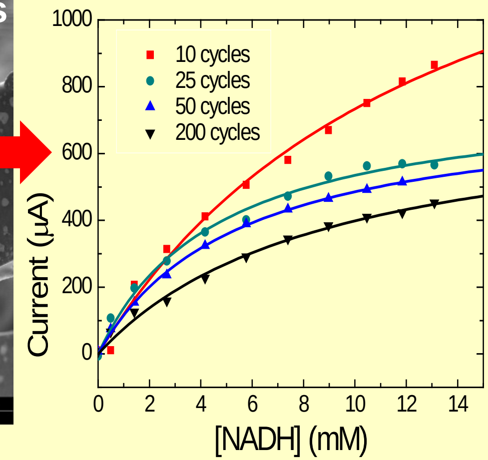
Deposition by cyclic voltammetry



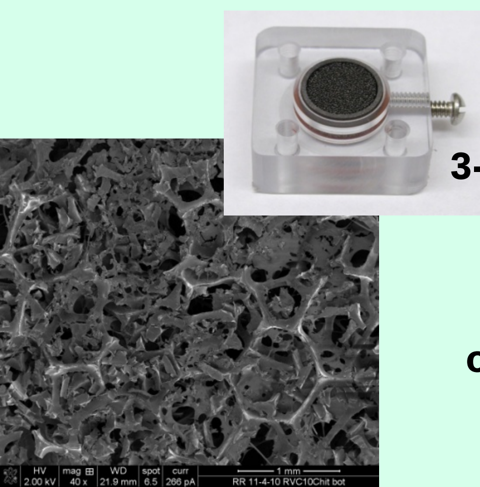




Electrochemical characterization



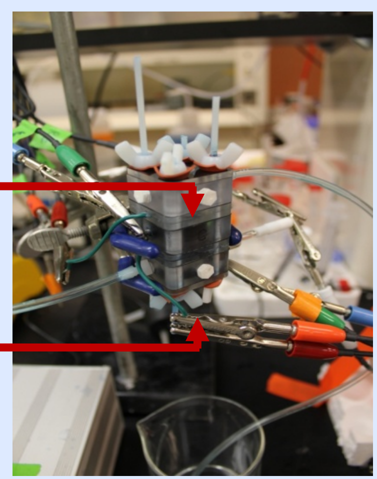
Integration of poly-(MG) modified RVC with NAD⁺-dependent enzymes immobilized in chitosan/CNTs composite scaffold



Integration with laccase-based bio-cathode in a flow-through membrane-less biofuel cell

3-D Anode

Cathode open to air

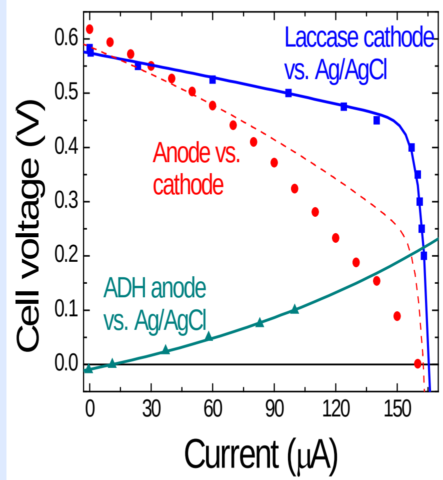


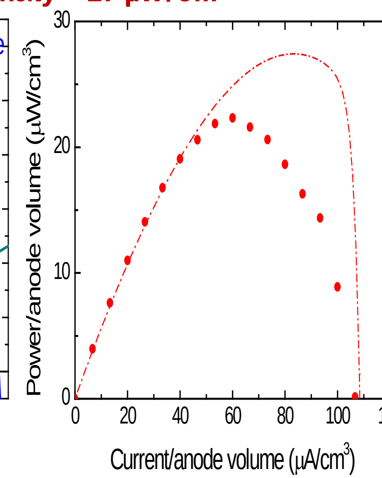
Polarization and power curves in 475 ethanol

$E_{0\ cell}$ = 0.618 V, pH = 6.3

Limiting current = 160 μA

Maximum power density = 27 μW/cm³





88 Personnel Involved in the Research: June 1, 2011

51 Supported by the MURI Program

6 University PIs and
8 Collaborators
88 Researchers involved

+ and 3 more…

51 of them supported fully or in part by the MURI

5 Research Faculty / Senior Researchers
18 Postdoctoral Fellows
34 Graduate Students
31 Undergraduate Students and 2 High School Students

36 Female

42 Male

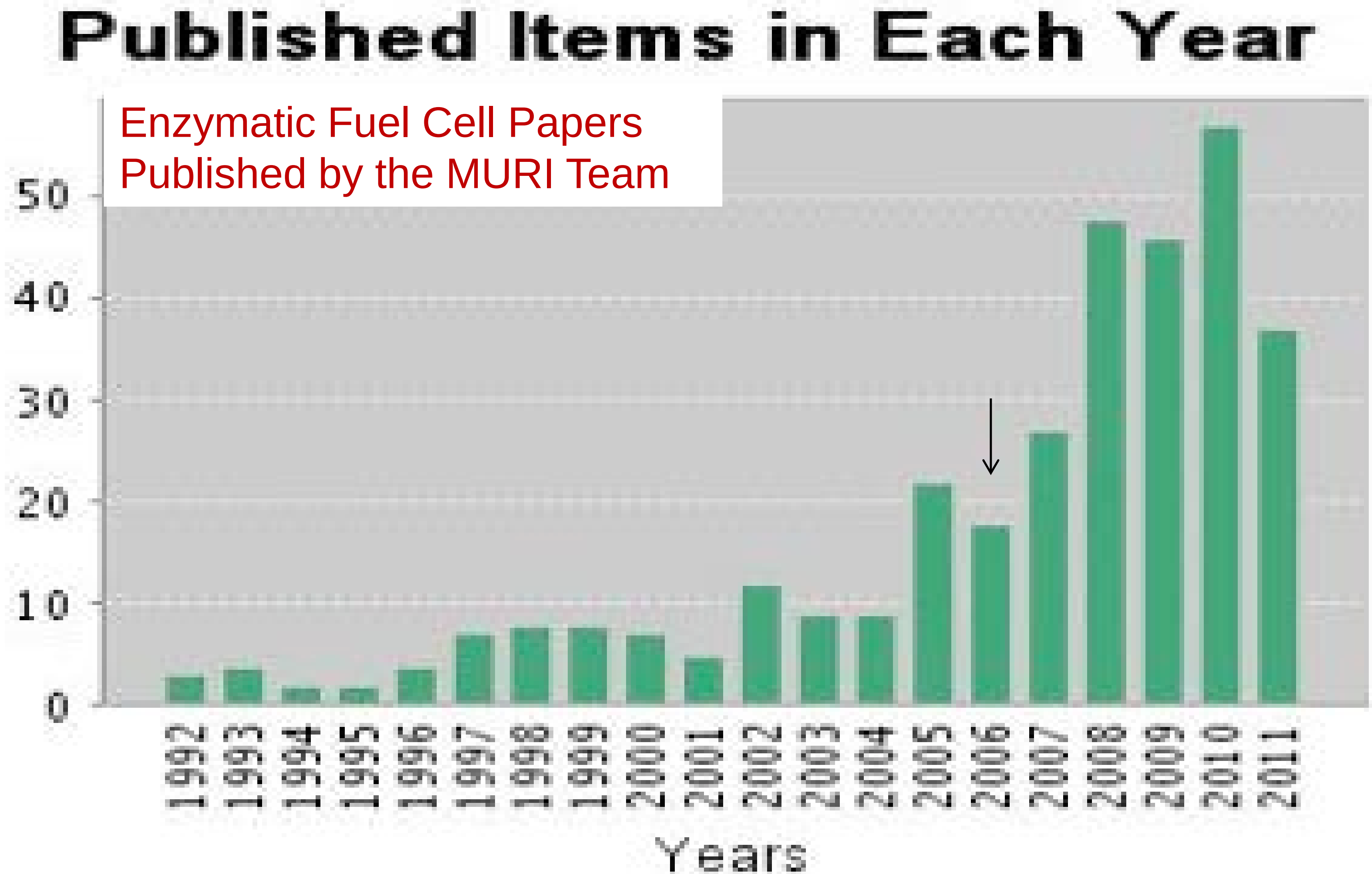
11 Hispanics

2 African American

1 Native American

ISI Publication Record on Enzymatic Fuel Cells: 1992 - 2011

AFOSR MURI: Fundamentals & Bioengineering of Enzyme Fuel Cells



Peer-Reviewed Journal Publications: June 1, 2011

99 Publications & Book Chapters and 6 Patent Applications

74 Published

16 Submitted or In Press

9 In Prep.

2010 Special Issue of Electroanalysis on Biofuel Cells

3 US Patent Applications

~ 215 Presentations at Conferences, With abstracts published in the Conference Proceedings, Including ~80 invited talks.

~ 75 Department Seminars, Press Releases, Interface article (ECS) Media Coverage, Issue Guest Editing.

Controlling Direct Electron Transfer (DET) Between Electrodes and Conductive Materials

Johnson & Pachter (AFRL) & Atanassov (UNM)

Objectives: Devise means to characterize and organize the interface between redox-active enzymes and nanomaterials

- **Background**: DET requires an electronic interface for electrons to "hop" from enzyme to the electrode surface.

  Multi-copper containing oxidases (MCO) serve as model bioelectrocatalysts for fuel cell cathode, accepting electrons from electrode and then catalyzing O_2 reduction.

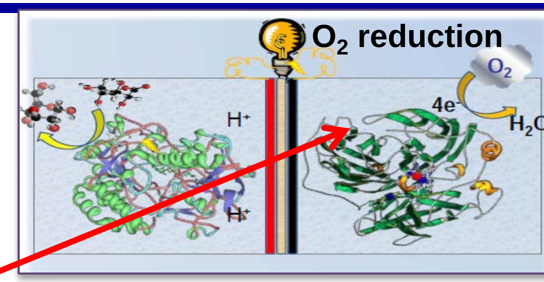
- **Approach**: Various MCO were linked to carbon nanotubes (CNT) using a chemical "tethering" reagent (1-pyrene butanoic acid, succinimidyl ester (**PBSE**)). The method conjugates the enzyme and CNT without changing material conductivity.

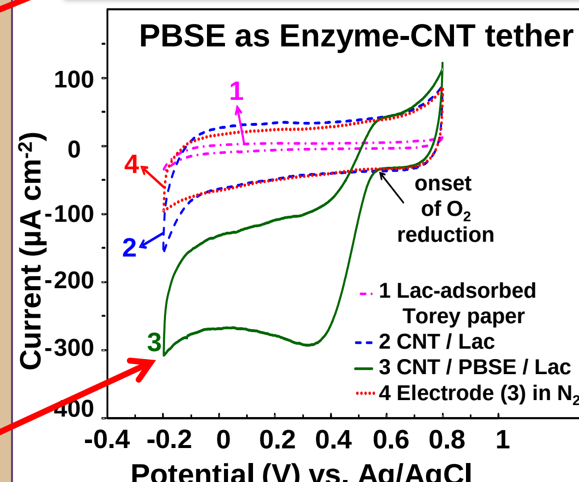
- **Results:** Electrochemical potential and kinetics of O_2 reduction reaction approach theoretical optima (+600 mV vs. Ag/AgCl)

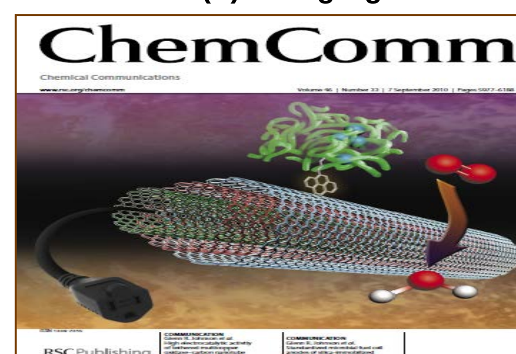
  High-potential maintained under increased current density, <100 mV decrease @ 50 mA cm^{-2}

  Bioelectrodes provided exceptional DET.

- **Conclusion**: Materials and processing approach accommodates various biocatalysts and is potentially scalable → significant advance over previous literature reports → key steps toward application. Cover feature on Chem Comm

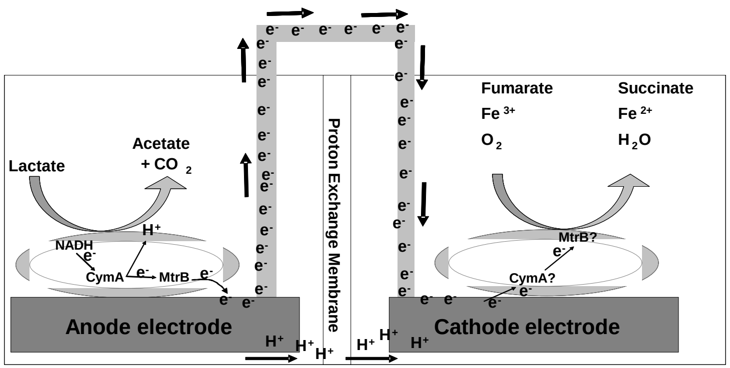






Chemical Communications 46:6045-6047

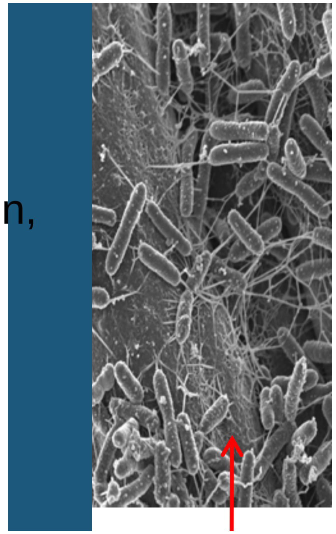
Microbial Fuel Cells
(MURI and Core Funding)



Optimizing Microbial Fuel Cells via Genetics, Modeling and Nanofabrication: Seven Labs

Objective:

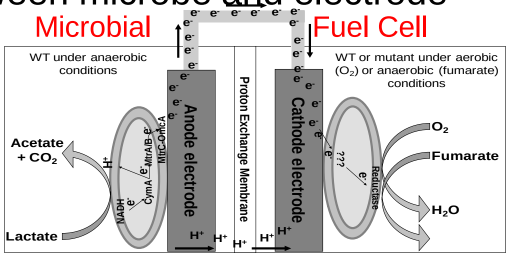
To understand the mechanism(s) involved in microbial current production, and to utilize multi-scale modeling to exploit this understanding in order to optimize microbes and microbial communities for microbial fuel cells.



Current transfer by nanowires…

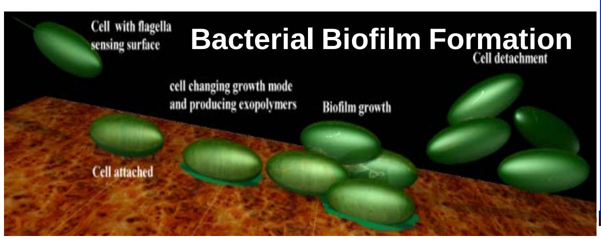
Technical Approach:

- Identification & regulation of the genes, molecular machines and structures used to produce and transfer current between microbe and electrode
- Modeling & bioengineering
- Development & exploitation of microbial consortia with the ability to utilize a wide range of energy sources
- Modeling, fabrication & testing of miniaturized MFCs

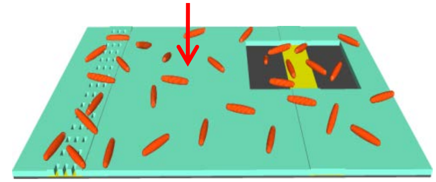


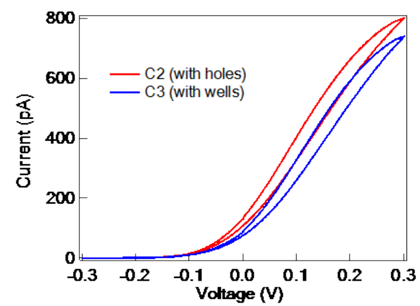
Accomplishments:

- Identified current associated genes in Shewanella
- Developed novel vertical scanning interferometry for interfacial analysis at electrode surface
- Characterized the bacterial behavior of electrokinesis
- Showed the value of bacterial biofilms in current production



…and/or soluble mediators?





DoD Benefit:

This project may enable high performance microbial fuel cells as power sources. The ability to use multiple complex fuels under changing physical and chemical conditions may enhance capabilities.

Molecular Identification of Bacterial Nanowires and Their Role in Microbial Fuel Cells: Ringeisen (NRL) Spring ReviewFY2012

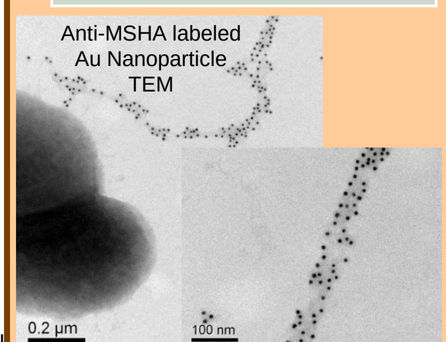
Objective: Use a variety of microbial fuel cell (MFC) platforms to correlate structure and function of extracellular nanofilaments with rate of extracellular electron transfer (current generation). Measure conductivity and protein identification of bacterial nanofilaments.

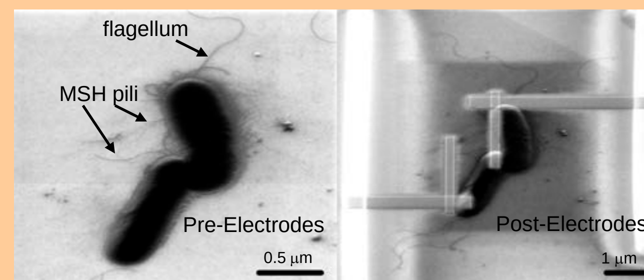
Technology Platforms Used for Protein ID of *Shewanella oneidensis* MR-1 Nanowires

- Miniature MFCs
- Direct Write Nanoelectrodes
- Immunolabeling and Transmission Electron Microscopy (TEM)
- Liquid Chromatography/Mass Spectrometry/Mass Spectrometry (LC/MS/MS)
- Temperature-Controlled Probe Station

Analysis of *S. oneidensis* nanofilaments has determined that a previously unsuspected protein (mannose sensitive haemagglutinin, MSH) is involved in extracellular electron transfer (EET) in microbial nanowires

Extracellular Protein ID in Nanofilament Preps via LC/MS/MS
MSHA
MSHB
Flagellin





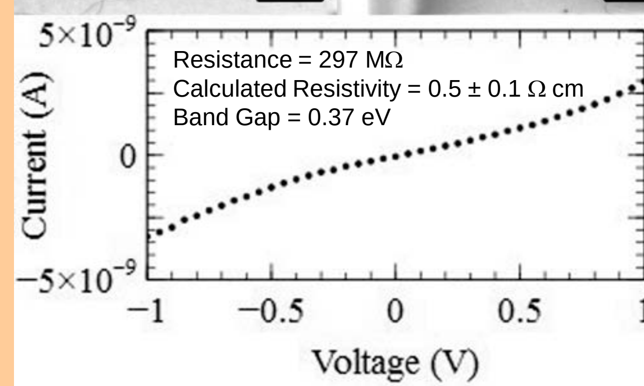


Photo-Electro-Magnetic Stimulation of Biological Responses (Core Funding)

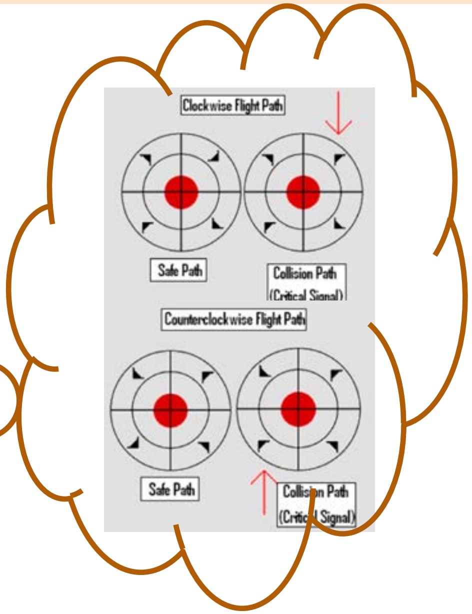
Photo-Electro-Magnetic Stimulation of Biological Responses is a beginning program that characterizes, models and explains the stimulatory and inhibitory responses of biological systems to low-level exposures of photo-electro-magnetic stimuli. Potential long-term benefits may include accelerated recovery from mental fatigue and drowsiness, enhanced learning and training, and noninvasive treatment of traumatic brain injuries. (~20% of portfolio)

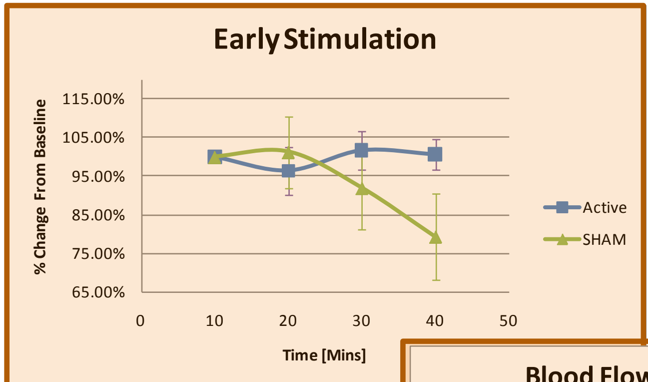
Electric Stimulation of the Brain, Hemodynamics and Sustained Attention:

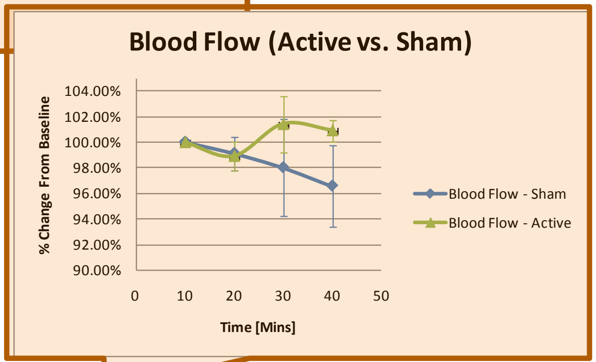
McKinley (AFRL/RH)

Objective: Quantify effects on human vigilance and hemodynamics due to non-invasive stimulation of the brain by low levels of direct current (1 mA).

NEW PROJECT 2011







Gordon et al., 2009

Astrocytes → rCBF

…?

Moore & Cao, 2008

Anodal Stim. → ↑ P(APs) → ↑ rSO_2 → ↑ Information processing → ↑ Vigilance Perform. → ↑ CO_2 → ↑ rCBF

Potential Metrics

Merzagora et al., 2010

Helton et al., 2010

Hellige, 1993 & Warm et al., 2009

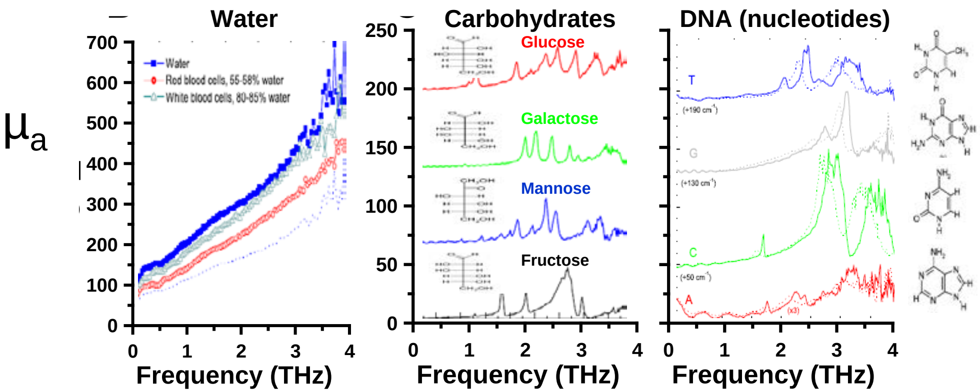
Coupling Terahertz Radiation to Biomolecules for Controlling Cell Response: Wilmink (AFRL/RHDR)

Terahertz (THz) Radiation:

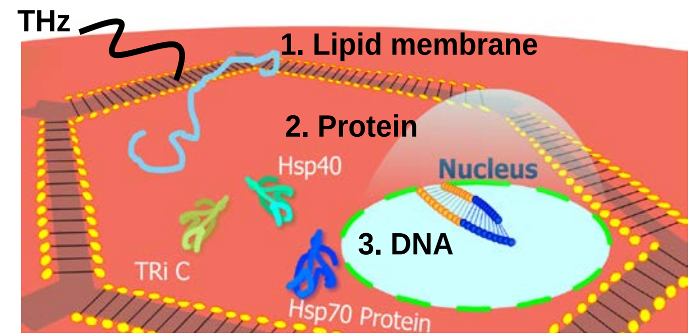
NEW PROJECT 2011

- Alters lipid membranes and modulates neuronal action potentials.
- Oscillates in the same ps time-scale as breathing modes of DNA & proteins (~40 ps).

Biomolecules display unique spectra in THz region

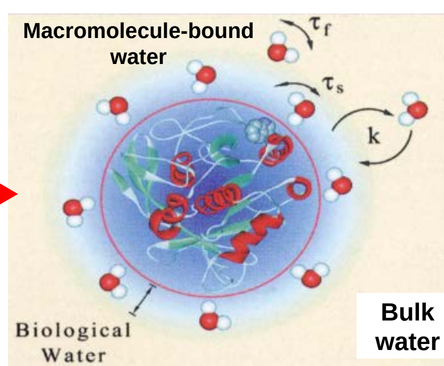


THz energy couples to biomolecules



Objectives: Investigate coupling mechanism and exploit the understanding to activate adaptive responses and modify cellular behaviors

Working Hypothesis: THz-coupling is mediated via macromolecule-bound water on the surface of membranes and biomolecules

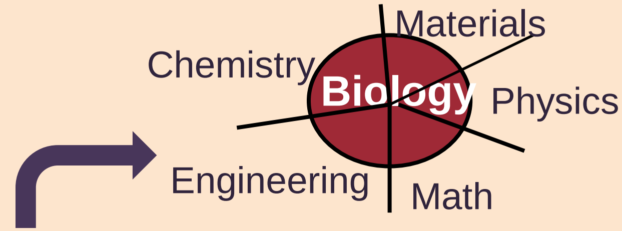


Testing Hypothesis:

- THz exposure system on a microscope
- Raman & THz spectroscopy
- Fluorescence & atomic force microscopy
- DNA mutation assays

Related Research Funded by Other Agencies

Funding Criteria:



1. Basic research of high quality and relevant to the AF
2. Unique or complementary, but non-duplicative—finds a "niche"
3. Leverages research in other agencies
4. Critical mass or team of collaborators with focused, multi-disciplinary research objectives

Algal Oil: DOE and DARPA research application oriented; NSF funds mostly individual grants of smaller size that are not based on a coordinated, multi-disciplinary team approach; USDA interested in farming aquaculture; EPA interested in regulation. AFOSR niche is lipid biosynthesis via systems biology. AFOSR has collaborated with DOE-NREL since 2006 and coordinates research as member of emerging Algal Interagency Working Group.

Biosolar Hydrogen: DOE and NSF fund mostly individual grants of smaller size that are not based on a coordinated, multi-disciplinary team approach. AFOSR niche is systems biology and bioengineering for enhanced H2 production. AFOSR has collaborated with DOE-NREL since 2003.

Biofuel Cells: ONR funds only microbial fuel cell (MFC) research for dissolved nutrients in the marine sediment environment. AFOSR funds enzymatic and MFC research for solid substrates in terrestrial environments and coordinates research via ONR reviews and direct personal contact.

Artificial Photosynthesis: This topic is biologically oriented and part of a 2009 AFOSR Initiative ***"Catalysts for Solar Fuels"*** with PMs Berman and Curcic, whose topics are chemically and physically oriented. To our knowledge there are no initiative counterparts at other agencies.

BioResponse to Photo-electromagnetic Stimulation: Complementary to other funded research.